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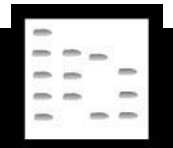
LIQUID CHROMATOGRAPHY-GAS CHROMATOGRAPHY



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Introduction

High resolution gas chromatography (HRGC) is the most suitable technique for the analysis of volatile compounds. If the sample is a complex matrix, such as natural products, food products or environmental pollutants, direct gas chromatographic (GC) analysis is not advisable for several reasons, and a sample pretreatment is necessary. In fact, peaks of different classes of compounds may overlap, rendering the qualitative and quantitative analysis of some compounds difficult. Moreover, if the compounds of interest are present only as trace amount, a preconcentration step is necessary before the GC analysis.

If the mixture is subjected to a preliminary separation by liquid chromatography (LC), the fraction so obtained can be analysed by GC. Offline coupling of LC and GC is laborious, involving numerous steps with the risk of contamination and possible greater loss of part of the sample than if online techniques were used. The online coupling of LC and GC offers a number of advantages compared to offline coupling: the amount of sample required is much lower; no sample work-up, evaporation or dilution is necessary and complex automated sample pretreatment is possible.

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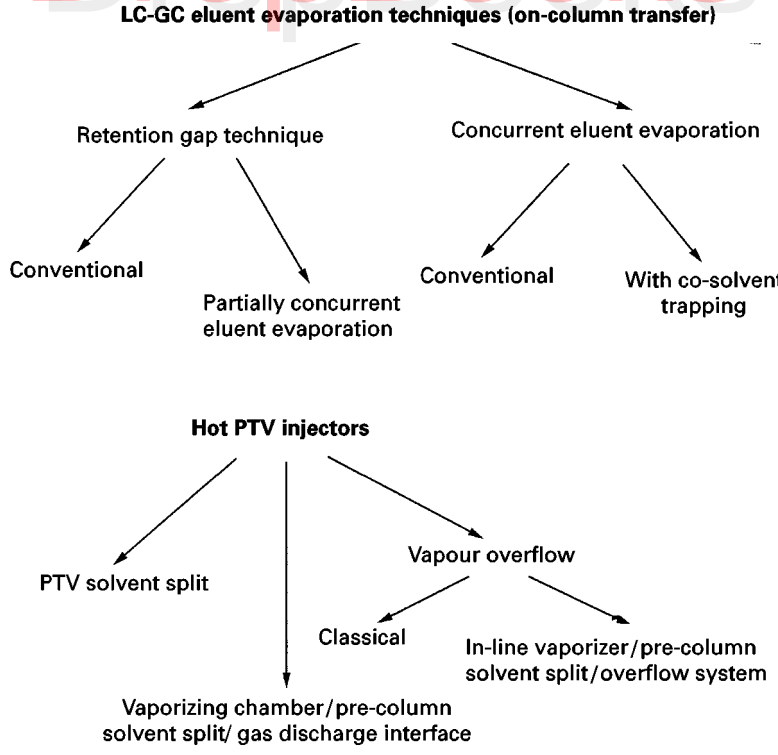

Figure 1 LC-GC transfer techniques. Possible mechanisms for LC-GC eluent evaporation.

The introduction of large amounts of solvent into a GC column requires the use of special techniques to separate the solvent from the sample selectively. At present, the principal techniques of eluent evaporation that allow transfer of large LC fraction into GC are based on the use of a modified on-column injector or on the use of a programmed temperature vaporizing (PTV) injector. **Figure 1** summarizes the transfer techniques used for online LC-GC coupling.

Concurrent Eluent Evaporation

Concurrent eluent evaporation is the most used technique, because of its simplicity and the possibility of transferring large amounts of solvent (up to 10 mL!). The technique involves complete evaporation of the eluent during its introduction into the GC system. This technique is suitable for the analysis of solutes with intermediate to high elution temperature, depending on the volatility of the eluent and on the volume of the LC fraction transferred.

When the sample solvent evaporates at the front end of the liquid, volatile compounds co-evaporate with the solvent and immediately start passing into the analytical column. The consequence is that, if the solvent vapours are vented through an early vapour exit, the volatile compounds are lost with the solvent vapour while, if venting is delayed, the most volatile compounds reach the detector even before the end of solvent evaporation. This causes a broadening of the initial band of solutes, which will give peaks that start with the solvent peak and spread out, masking some of the volatile peaks. Only high boiling substances, which migrate slowly during the transfer, will elute after the oven temperature increase, and will give sharp peaks. In practice, the first properly shaped peaks are eluted some 40–120°C above the transfer temperature.

The fraction to be analysed is contained in a loop, connected to a switching valve. The opening of the valve allows the sample in the loop to be driven by the carrier gas into the GC. Usually an early vapour exit is located after a few metres of deactivated precolumn and 3–4 m of retaining column (cut from the analytical column). The retaining pre-column provides a short zone where the solute can be focused in the stationary phase, so that volatile components will be trapped, avoiding their loss through the vapour exit. This is opened during solvent evaporation to reduce the amount of solvent that would reach the detector, and at the same time to increase the solvent evaporation rate.

Figure 2 shows the scheme of the loop-type interface.

Figure 3 shows an example of tocopherols, free sterols and esterified sterols determined in a single analytical run after acetylation. These compounds were pre-separated from the triacylglycerols by normal-phase LC.

Retention Gap

The retention gap method represents the best approach in the case of qualitative and quantitative analysis of samples containing highly volatile compounds. In fact, the retention gap technique allows the analysis of substances eluting immediately after the solvent peak, due to the reconcentration of these components by the so-called solvent effects (primarily solvent trapping). **Figure 4** shows the scheme of the retention gap transfer technique.

The term retention gap means a column inlet of a retention power lower than that of the analytical column. In the retention gap technique, the sample is introduced into the GC at a temperature below the boiling point of the LC eluent (corrected for the current inlet pressure). In this way, the solvent

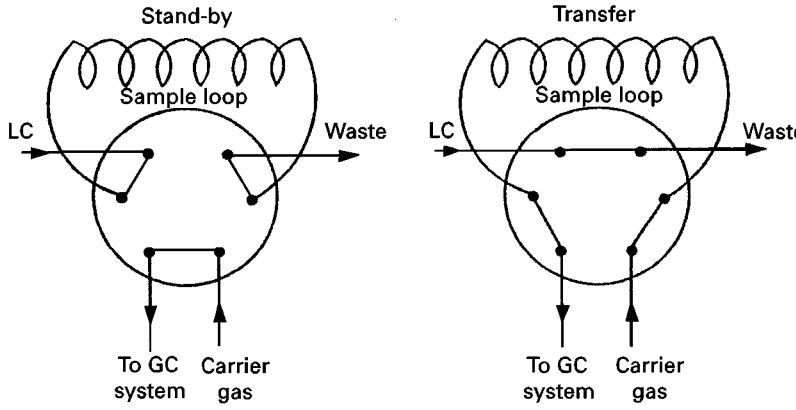

Figure 2 Loop interface scheme for concurrent eluent evaporation.

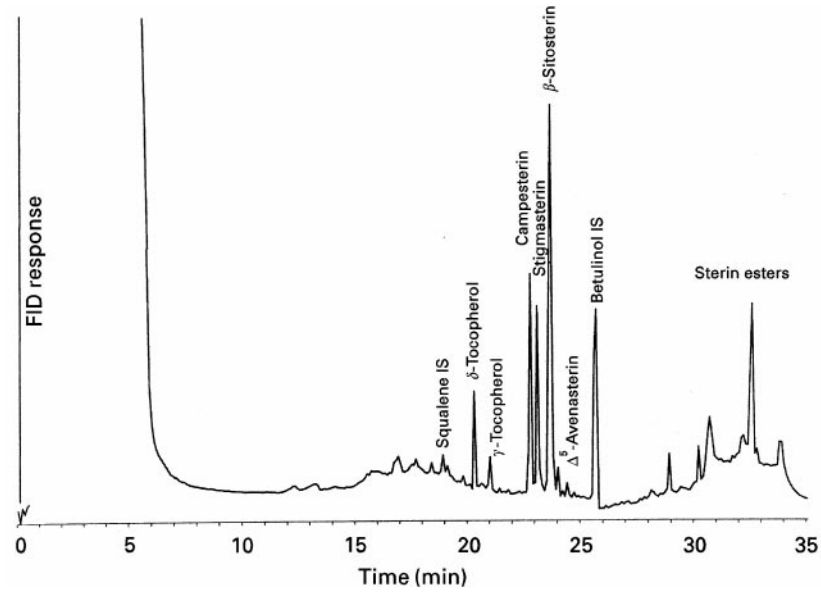

Figure 3 GC chromatogram of tocopherols, free sterols and esterified sterols transferred from the LC pre-separation system using the concurrent solvent evaporation technique. FID, Flame ionization detector. (Reproduced with permission from Lechner M and Lorber E (1998) *20th International Symposium on Capillary Chromatography*, Riva del Garda, Italy. Copyright P. Sandra and AJ Rackstraw.)

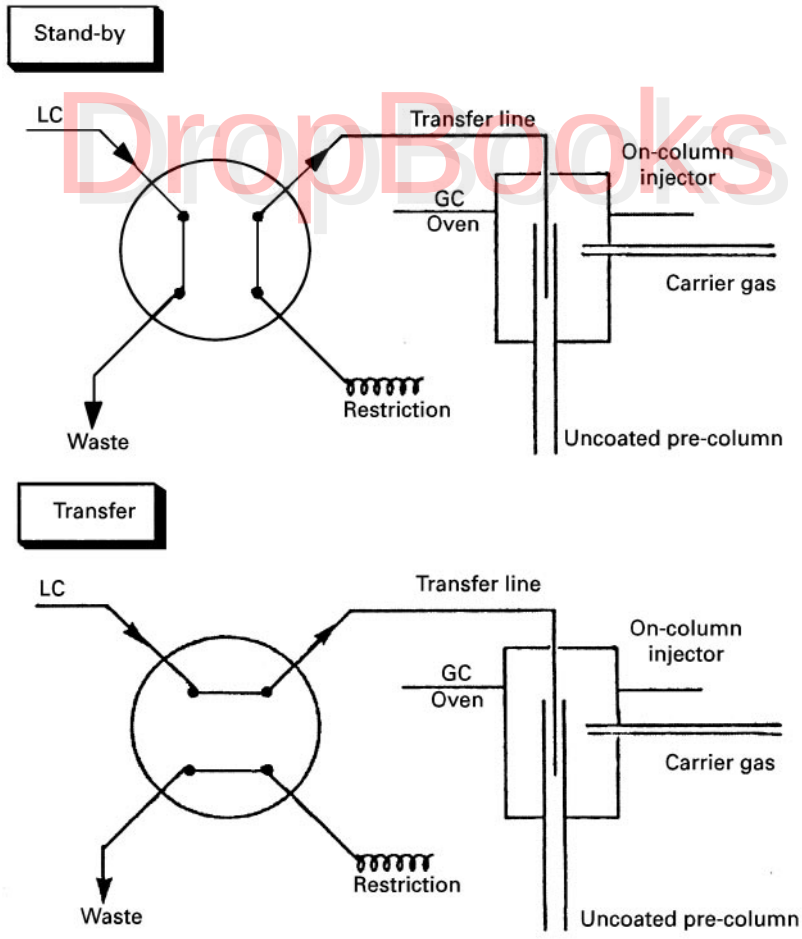

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Figure 4 Retention gap scheme.

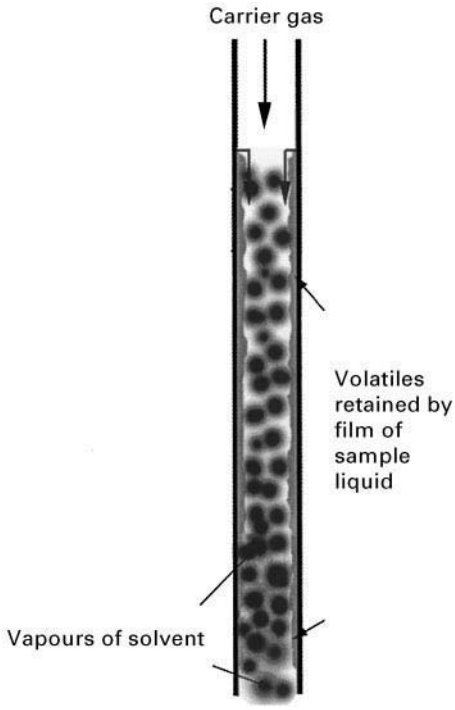

Figure 5 Solvent evaporation in a retention gap. The evaporation occurs from the rear part of the solvent.

vapours replace only part of the carrier gas, which continues to flow through the column. In the carrier stream, solvent evaporates from the rear towards the front of the sample layer (**Figure 5**). Under these conditions, the volatile compounds that are liberated from the solvent envelope start moving, but they are immediately trapped again by the solvent present ahead of the evaporation site (solvent-trapping effect). In this way, the volatile components can start moving in the carrier gas only when the process of solvent evaporation is complete.

A second effect, called phase soaking, occurs in the retention gap technique. This effect is obtained because the carrier gas is saturated by solvent vapours, so it swells the stationary phase with this amount of solvent. The consequence is that the column shows an increased retention power that can be used to trap the volatile compounds.

During the evaporation process, band broadening in space spreads the high boiling compounds. Two retention gap effects can reconcentrate these solute bands. First, compounds migrate more rapidly through the retention gap zone than through the main column; reconcentration depends on the ratio between the retention power in the pre-column and in the main column (phase-ratio focusing). Second, the compounds arrive at the entrance of the main column at a temperature at which they are practically unable to migrate, so they accumulate until the temperature is increased, and concentrate. The limitation of the method is that, due to the poor capacity of uncoated pre-columns to retain liquid, only modest volumes can be transferred (100–150 μL of eluent) and long uncoated pre-columns are needed.

Two additional techniques have been developed to overcome the drawbacks of the techniques described above, with the aim of obtaining sharp peaks at elution temperatures below 120–150°C, for concurrent eluent evaporation, and to transfer large LC fractions, for the retention gap technique: partially concurrent solvent evaporation and co-solvent trapping.

Partially Concurrent Solvent Evaporation

Partially concurrent solvent evaporation allows working under conditions that still produce a zone flooded by the eluent (retention gap), providing solvent trapping. This causes a large amount of eluent to evaporate during its introduction (concurrently), so that shorter uncoated pre-columns or larger volumes of transferred fraction can be handled. In practice, an early vapour vent may be placed between the uncoated pre-column and the analytical column, but this makes closure of the vent critical for partial losses of early eluted peaks. A section of the analytical column may be installed after the uncoated pre-column but before the solvent vapour exit.

Figure 6 shows GC chromatograms obtained after pre-separation by normal-phase LC, applying the partially concurrent solvent evaporation. This application consists of the measurement of the enantiomeric ratio of linalol contained in sweet and bitter orange essential oil. This ratio characterizes each oil, differentiating them, so that the presence of the less valuable sweet orange oil can be detected in the more valuable bitter orange oil.

Co-solvent Trapping

Co-solvent trapping is used to obtain sharp solute peaks of correct size even for volatile compounds, with concurrent evaporation technique. A small amount of a higher boiling co-solvent is added to the main solvent (for example, *n*-heptane added to pentane), to prevent co-evaporation of the volatile compounds with the main solvent (**Figure 7**).

It is necessary to adjust the concentration of co-solvent so that some co-solvent is left behind as a liquid, while some is evaporated with the main solvent. In this way, the co-solvent remaining forms a layer of liquid film that evaporates from the rear to the front, as in the retention gap technique. The solutes are trapped by the solvent-trapping effect, and

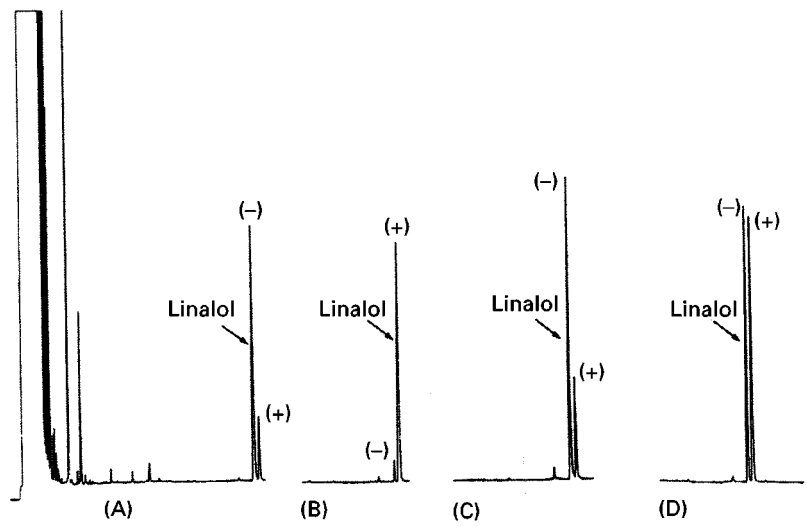

Figure 6 GC chiral separation of linalol from sweet orange, bitter orange and mixtures of the two essential oils, showing the different enantiomeric ratios. Linalol fraction was transferred from the LC system using the partially concurrent solvent evaporation technique. (A) Bitter orange; (B) sweet orange; (C) bitter orange 90%, sweet orange 10%; (D) bitter orange 70%, sweet orange 30%. (Reproduced with permission from Dugo G, Venzera A, Cotroneo A, Stagno d'Alcontres W, Mondello L and Bartle KD (1994) *Flavour and Fragnance Journal* 9: 99. Copyright John Wiley & Sons Limited.)

are released after the co-solvent has been evaporated. As can be seen, this technique shows some similarity to partially concurrent solvent evaporation. The main difference is that it is designed for the loop-type interface, while partially concurrent solvent evaporation works with an on-column interface. The optimization of the transfer conditions is not easy, so this technique has never been routinely applied to the transfer of normal-phase eluents, because the partially concurrent solvent evaporation technique is easier to use. It can be the technique of choice for the transfer of water-containing solvents, because in contrast to retention gap techniques, concurrent eluent evaporation does not need wettability, and co-solvent trapping retains the volatile solute. Under these conditions, transfer occurs at 110–120°C, so the first compounds should elute at this temperature. Unfortunately, the problem is the lack of an uncoated pre-column resistant to condensed water. Studies on improving pre-column deactivation have been carried out, but the scant interest in reversed-phase LC coupled to GC prevented re-evaluation of this promising technique with improved methods of deactivation.

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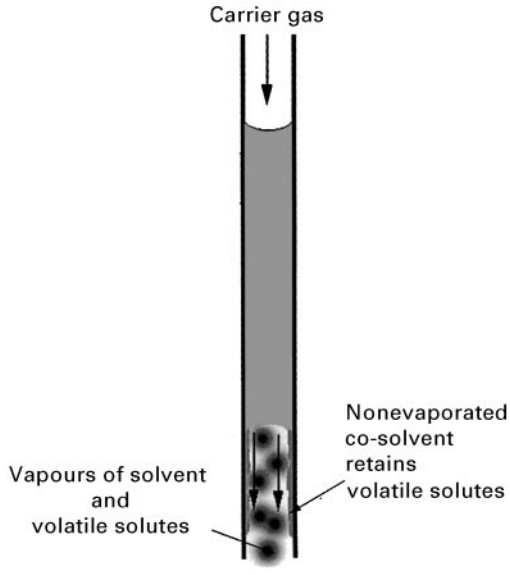

Figure 7 Concurrent eluent evaporation with co-solvent trapping effect. The volatile components are retained by the solvent film.

Vaporization with Hot Injectors

Together with the techniques described above, other techniques using hot injectors for the transfer from LC to GC, have been developed. PTV with solvent trapping in packed beds can be used successfully to transfer large volume fractions into the GC instead of into a capillary pre-column (uncoated, deactivated silica tubing). This technique shows some advantages compared to the techniques that use on-column sample introduction:

1. Packed beds retain more liquid per unit internal volume.
2. Wettability is no longer critical.
3. Packing material is more stable than deactivated silica tubing.
4. PTV is more easily heated than capillary column.

Because of the high retention power, the packed chambers have to be heated above the column temperature to release the solutes. This is a drawback in the analysis of thermally labile compounds, and high boiling compounds. In the PTV solvent split mode, the injection is made into a cool injector, and the solvent is largely removed via the split valve. Volatile compounds can evaporate with the solvent; many studies have been carried out to minimize the loss of volatiles, for example, reducing the temperature of the injector. On the other hand, high boiling compounds are difficult to release, due to the high retention power of the packing material. Introduction of large volumes in the PTV solvent-split mode was introduced in the 1970s and, since its introduction, many different approaches have been described.

The first configuration was a glass tube with a packed bed with a cool injector. The solvent vapours are discharged through the split valve. After solvent evaporation the split valve is closed and the injection chamber is heated to transfer the solutes into the column. Liners are usually packed with Tenax TA and Thermotrap TA. To increase trapping efficiency, liners with sintered porous glass beads were introduced.

Recently a new, fully automated online LC-GC coupling has been introduced. This interface consists of a flow cell, where the fraction is sampled by a large volume autosampler, and automatically injected into a PTV device using the solvent vent mode. This technique was successfully applied to determine pesticide residues in complex natural matrices such as essential oils (**Figure 8**).

Vapour Overflow

The vapour overflow technique is intended for introducing samples into large volumes of solvent by syringe injection of dilute samples or by coupled LC-GC. The liquid is introduced into a packed (generally with Tenax) vaporizing chamber maintained above the solvent boiling point at a pressure which is near or below ambient. This technique is performed in the absence of carrier gas and vapours are discharged by expansion during evaporation (overflow). The vaporizing chamber is filled with packing material of a GC retention power for the volatile components. The carrier gas supply line is equipped with a switching valve, allowing the gas supply to be stopped during sample introduction. The sample is released by a syringe or a transfer line from the LC near the bottom of the voporizing chamber. Vapours expand and, driven by the expansion, leave the system through the septum purge (**Figure 9**).

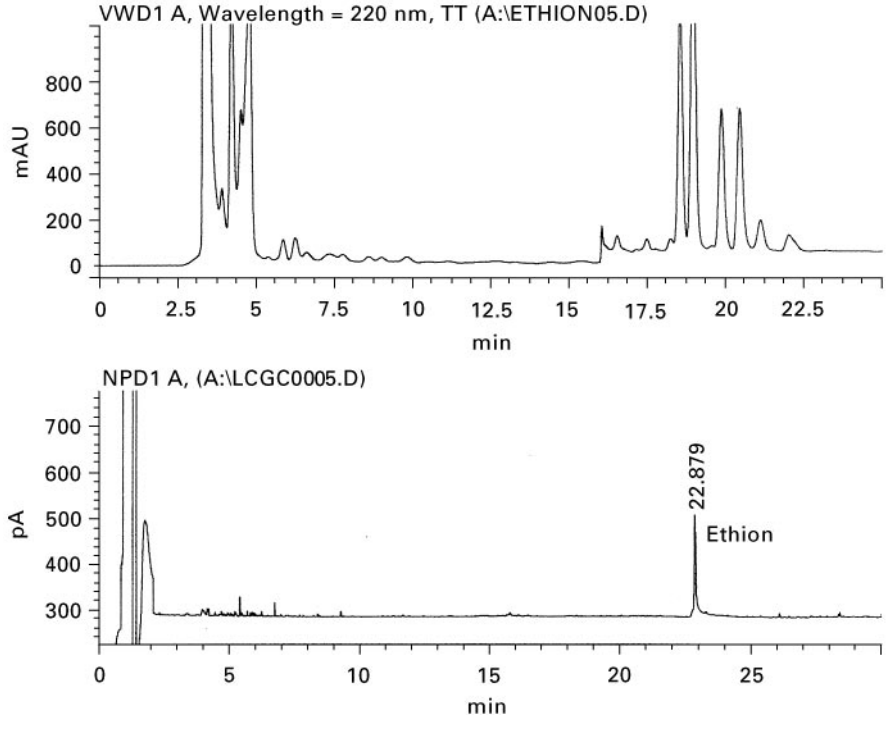

Figure 8 LC separation of (A) orange essential oil and (B) GC/NPD chromatogram of the fraction containing the pesticide ethion, transferred from the LC system, using the PTV interface type in the solvent vent mode. NPD, nitorgen-phosphorous detector. (Reproduced with permission from David F, Correa RC and Sandra P (1998) *20th International Symposium on Capillary Chromatography*, Riva del Garda, Italy. Copyright P. Sandra and AJ Rackshaw.)

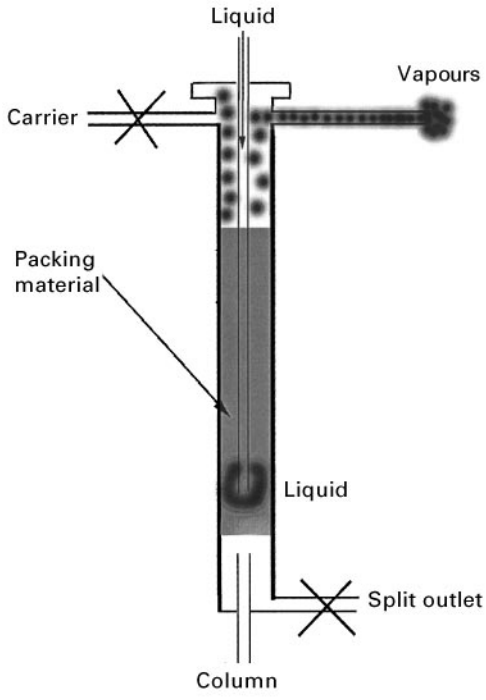

Figure 9 Vapour overflow interface. The solvent vapours are eliminated through the septum purge of the PTV injector.

After solvent evaporation is completed, the septum purge is closed, the injector is warmed up (PTV) and the trapped analytes are released into the column. More recently, this system has been modified for use with a conventional hot injector.

In-line Vaporizer/Pre-column Solvent-split/Overflow System

The in-line vaporizer/pre-column solvent-split/overflow system was developed by Grob for large volume liquid injection and for online LC-GC. This system is an overflow-based technique, but with an improvement for the retention of more volatile compounds. As can be seen from **Figure 10**, the vaporizer consisted of a transfer line (from the LC) of fused silica. Inside the vaporizer a 5 cm length of steel wire (untreated or deactivated) or of fused silica is inserted for complete evaporation of the liquid (250–350°C). Moreover the system is equipped with a retaining pre-column and an early vapour exit. The oven in this case is maintained at a low temperature (lower than with a loop interface) to improve retention of sample components by phase soaking. The improvement in the arrangement corresponds to about four extra carbon atoms retained (undecane instead of pentadecane).

Vaporizing Chamber/Pre-column Solvent-split/Gas Discharge Interface

The sample is injected by an autosampler or by online transfer from a high performance liquid chromatography (HPLC) into a heated device. The vaporizer is packed and maintained at a temperature suitable for solute evaporation. Compounds that are sensitive to high temperatures are injected into a PTV injector in

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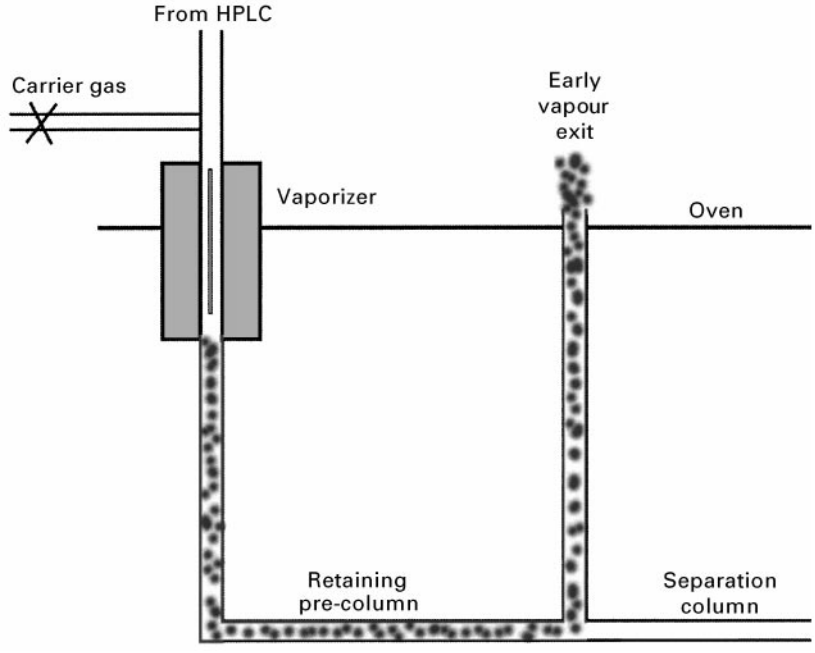

Figure 10 In line vaporizer/pre-column solvent-split/overflow system. The more volatile components are retained by the retention gap, letting the solvent out from the early vapour exit.

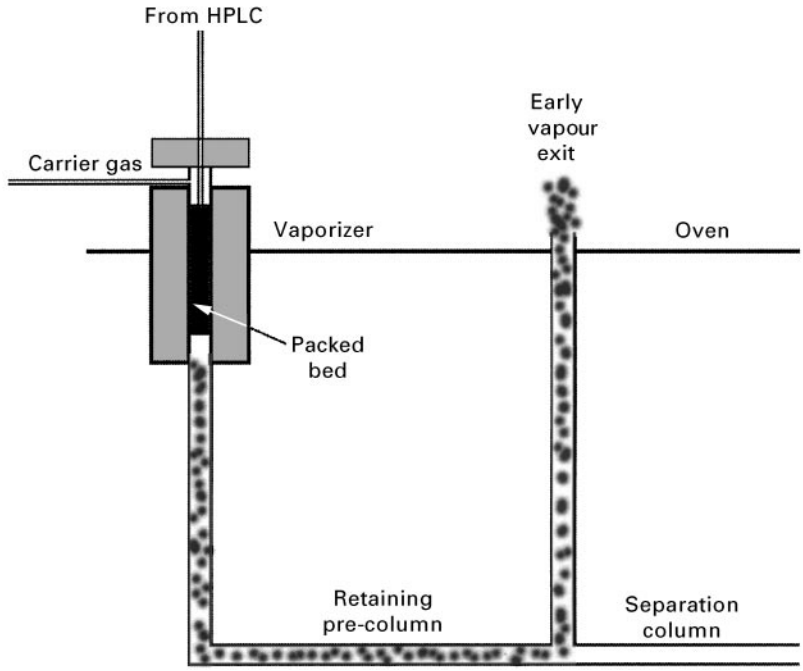

Figure 11 Vaporizing chamber/pre-column solvent-split/gas discharge interface. The vaporizer is packed and heated at a suitable temperature for solvent evaporation. The vapour exit can be positioned at the end of the retention gap.

order to evaporate the solvent first and then the solutes. As shown in **Figure 11**, the vapours are discharged through a pre-column and an early vapour exit. If solvent trapping is needed, an uncoated pre-column is installed after the injector and before the solvent vapour exit. Moreover, the use of a retaining pre-column is possible depending on how critical is the solvent vapour exit closure.

Conclusions

Coupled LC-GC is an excellent online method for sample preparation and clean-up. For the analysis of nonaqueous media the transfer technique of choice is certainly concurrent eluent evaporation using a loop-type interface. The main drawback of this technique is the loss of volatile solutes due to the solute co-evaporation with the solvent. If volatile compounds are present, transfer from LC to GC is best achieved by a retention gap technique. This on-column method yields sharp peaks starting from the programming of the GC oven temperature during eluent transfer. However, due to the limited capacity of uncoated pre-columns for liquid retention, the technique is only suited for the transfer of relatively small fractions. Larger fractions can be transferred by partially concurrent eluent evaporation since this includes the retention gap procedure. Presently, PTV solvent-split injection is considered as the method of choice for large volume injection of 'dirty' or water-containing samples. Finally, vaporizing chamber/pre-column solvent-split/gas discharge interface and in-line vaporizer/pre-column solvent-split/overflow systems seem to be promising techniques for LC-GC coupling in a wide range of applications.

See also: **I/Chromatography. II/Chromatography: Gas** Column Technology: Multidimensional Gas Chromatography; Theory of Gas Chromatography. **Chromatography: Liquid:** Multidimensional Chromatography; Theory of Liquid Chromatography.

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